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Detection of plasmin based on specific peptide substrate using acoustic transducer

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ABSTRACT

In this work we report the detection of plasmin protease by means of the thickness shear mode (TSM) acoustic method. The biorecognition element consists of a peptide substrate (PS) specific to plasmin immobilized on a piezoelectric quartz crystal electrode. After enzymatic reaction with plasmin, it cleaves a short fragment of the peptide causing increase in the resonance frequency of the piezo crystal. Plasmin was detected in the range of concentrations 1–20 nM, a target interval in which its presence presumably affects the quality of milk. The PS exhibited negligible response against to similar protease trypsin. This has been confirmed also by electrochemical detection method. Limit of detection of this acoustic transducer was found to be 0.65 nM. Formation of the sensing surface and kinetic effect of plasmin on the peptide substrate was studied by atomic force microscopy (AFM). The PS response was also validated in pretreated milk samples spiked by known concentrations of plasmin achieving an average recovery of 63 ± 0.6%.

1. Introduction

Proteolytic activities in milk comprise both, positive and negative impacts on industrial applications. For instance, it has been demonstrated that degradation of milk proteins highly influences cheese ripening through development of desirable changes in flavor and consistency. In contrast, proteolysis is undesirable when it results in gelation and bitterness due casein breakdown as observed in ultra high temperature (UHT) treated milk [1]. Proteolysis of milk has been connected to the natural serine protein plasmin, the most abundant endogenous protease transferred from blood into bovine milk [2]. Despite of the quantification of plasmin activity in dairy products could have important industrial impacts, not enough information has been yet provided about the amounts of plasmin present and its effect on the properties of the product. Additionally, since plasmin is a part of an intricate enzymatic-inhibitory system its detection is not a simple task. Factors such as cow characteristics, processing conditions, other milk components, storage conditions, bacterial proteases and mutual interaction of other components of the plasmin system could compromise the accurate and effective detection of this protease [3].

Up to date, plasmin has been mainly detected by immunological and spectroscopic methods [4]. Separation techniques such as reversed-phase HPLC [5], gel electrophoresis [6], tri-nitro-benzene-sulfonic acid (TNBS) [7] and fluorometric methods [8] have also been reported. Fluorescence technique based on fluorescamine has the advantage of the lowest detection limit compared with the other methods, whereas gel electrophoresis has shown to be the best qualitative method; however none of these is as sensitive as HPLC. The TNBS method is recommended for use in routine laboratory analysis on the basis of its accuracy, reliability and simplicity, but lacks high sensitivity and exhibits low limit of detection [7]. Recently, a peptide substrate (PS) consisting of four amino acids specifically synthesized for cleavage of plasmin has been implemented as biorecognition element in electrochemical and photometric biosensors [9,10]. Although highly effective and sensitive, these methods are not completely adaptable to in situ and real time conditions usually required when analyzing food samples. Furthermore, confrontation with other techniques

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